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Cancer metabolism

Losing control of nutrient sensing in the germinal center drives lymphomagenesis

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The gene encoding RagC GTPase (*RRAGC*), an activator of a nutrient-sensing pathway that drives cellular anabolism, is mutated in 15% of follicular lymphoma cases. A new study provides evidence that *RRAGC* mutations promote lymphomagenesis by distorting a nutrient-dependent control of paracrine signals from the microenvironment, resulting in enhanced B-cell activation.

Follicular lymphoma (FL) is an indolent but largely incurable non-Hodgkin lymphoma originating from the clonal expansion of germinal center (GC) B-cells,[1] a specialized subset of B-cells that during a T cell-dependent immune response modify their rearranged immunoglobulin variable region genes to generate highly specific, pathogen-eliminating antibodies.[2,3] The genetic hallmark of FL is the t(14;18) chromosomal translocation that constitutively activates the anti-apoptotic gene *BCL2*, along with recurrent mutations of histone modifier genes.[1] Additional genetic mutations affecting various biological pathways have been identified by genomic analyses,[1,4] including aberrations in components of the cellular nutrient-sensing pathway. Specifically, a

sizable subset of FL cases show missense mutations in the nucleotide binding domain of the gene encoding the RagC GTPase (*RRAGC*).[5,6] This enzyme forms a heterodimeric complex together with RagA GTPase, which activates mTORC1 when cellular nutrients are sufficient, leading to cell growth.[7,8] Curiously, RagA, despite encompassing a part of the mTORC1-activating complex, is not targeted by mutations in FL.[5,6] These observations raised two questions: *1)* how do mutations in *RRAGC* promote FL pathogenesis; and *2)* what is the basis for the selectivity of mutations in *RRAGC*, while sparing RagA? Through mimicking the FL-associated *RRAGC* mutations in the mouse germline and crossing those mice to an established FL animal model, Ortega-Molina et al. uncovered a role for RagC in normal GC B-cell development and FL pathogenesis.[9]

Employing CRISPR/Cas9 genome engineering, Ortega-Molina et al. generated two independent *Rragc* knockin-mouse models that mimic the most frequent human variants of activating *RRAGC* mutations.[9] In order to assess the presumed aberrant activity of mutated RagC *in vitro*, amino acids and growth factors that would elicit mTORC1 activation were omitted from the culture medium. Under these conditions, B-cells of the mutant mouse lines showed a greater mTORC1 activity than wild-type mice upon T cell-mediated activating signals that during an immune response stimulate the mTORC1 pathway via the PI3K-AKT axis (Fig. 1A), suggesting a partial insensitivity to nutrient withdrawal (Fig. 1B). The same was observed on a *Bcl2*-transgenic background using *VavP-Bcl2* transgenic mice,[10] which also revealed accelerated FL development *in vivo*.[9] Transcriptional profiling analysis showed enrichment of the mTORC1 signature in RagC-mutated FL cells, and a marked overlap in the differential expression of the corresponding genes in *RRAGC*–mutated murine and human FL. These results suggest that RagC-mediated enhancement of mTORC1 activation promotes FL pathogenesis also in humans.

In order to gain insights into the pathogenic mechanism of mutated RagC in FL development, the authors determined the biological effects of the mutated gene in the normal cellular counterpart of FL. Upon immunizing RagC-mutated mice with a T cell-dependent antigen, the authors observed a dramatic increase in the abundance of GC B-cells compared to wild-type controls.[9] The difference was even more remarkable in a competitive reconstitution setting where RagC mutant and wild-type B-cells were assessed in their ability to generate GCs in the same mouse. Mechanistically, the authors provide evidence that this increase in RagC mutant cells is likely due to suppression of cell death and to a decreased requirement of the RagC-mutated GC B-cells on microenvironmental signals provided by T-follicular helper (Tfh) cells, the $CD4^+$ T-cells that control the GC reaction. The latter was evident from the observation that despite the massive enlargement of GCs in the RagC-mutated mice, the number of Tfh-cells per GC was similar to that of control mice. Since Tfh-cells are required for GC maintenance, the decrease in the Tfh/GC B-cell ratio suggests that *Rragc* mutations promote lymphomagenesis by substituting PI3K-AKT-derived mTORC1-activating signals through the Rag GTPase-mediated nutrient-sensing pathway (Fig. 1B).

Now, if aberrant Rag GTPase function promotes FL pathogenesis via mTORC1 activation, why are no activating mutations observed in RagC's heterodimeric partner RagA? Ortega-Molina et al. provide evidence for what they call a 'biochemical asymmetry' of the Rag heterodimers in the activation of mTORC1. Unlike observed for mutated RagC that was sensitive to nutrient withdrawal, an activating mutation in RagA had no effect on the regulation of mTORC1 under the same condition.[9] Moreover, the RagA mutation did not lead to GC enlargement, and constitutive activation of RagA in GC B-cells actually impaired the GC response by decreasing GC B-cell fitness, similar to

what has previously been observed for mTORC1 hyperactivation.[11] Specifically, strong mTORC1 activation negatively impacted the generation of high-affinity antibodies against the immunizing antigen, which in a competitive setting led to the disappearance of mTORC1-hyperactive versus wild-type GC B-cells over time.[11] Conversely, mutations in RagC were found to increase the production of high-affinity antibodies.[8] Based on these findings, the authors propose a model in which the mutations in RagC, as opposed to those in RagA, lead to a modest activation of the mTORC1 pathway that increases GC B-cell fitness (Fig. 1B).

But how would an increased fitness of RagC-mutated GC B-cells contribute to the multistep process of FL development? Adding to the pro-survival signals provided by the BCL2 translocation, RagC-induced mTORC1 activation may further suppress the default apoptotic program of GC B-cells by enhancing activating Tfh cell-derived signals, which also funnel into the mTORC pathway. This pre-malignant GC B-cell has a competitive advantage over normal GC B-cells with similar antigen-affinities and keeps undergoing iterative cycles of selection and proliferation within the GC, during which it may acquire additional genetic aberrations, ultimately developing into a *bone fide* lymphoma.

Then the pressing question is whether enhanced mTORC1 activity in FL with *Rragc* mutations and *Bcl2*-translocations can be exploited for lymphoma therapy. Experiments performed by Ortega-Molina et al. with the mTOR inhibitor rapamycin indicate that such mice, in contrast to mice with *Bcl2*-translocations only, showed a lower incidence and grade of lymphomas.[9] The observed higher selective sensitivity to mTORC1 inhibition in this mouse model suggests that patients with mutations in the nutrient-signaling pathway may benefit from treatment with mTOR inhibitors.

Finally, the authors propose that a different genetic aberration in FL may act in a similar manner as *RRAGC* mutations. Loss-of-function mutations or deletions of *TNFRSF14* that ablate the function of Herpes virus entry mediator (HVEM) are associated with an increased cellularity of Tfh-cells in the tumor microenvironment.[12] A meta-analysis of genomic data from human FL samples revealed that *TNFRSF14* mutations are largely mutually exclusive with *RRAGC* mutations.[9] Intriguingly, a recent publication reports that HVEM deficiency was associated with increased B-cell competitiveness during the GC reaction,[13] mirroring what Ortega-Molina et al. observed for RagC-mutated B-cells. Therefore, it seems that Tfh cell-derived signals may promote lymphoma growth either by cell-autonomous mutations that synergize with those signals, as observed for *RRAGC* mutations, or by abnormally high Tfh-cell numbers as in the case of *TNFRSF14* deficiency. The findings by Ortega-Molina et al. provide important new insights into the molecular mechanisms of FL pathogenesis. In future studies, the potential tumor metabolic vulnerability identified in the present work may be exploited for the development of precision medicine-based therapies.

Competing interests

The author declares no competing interests.

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Figure Legend

Figure 1: Model for the effects of RagC mutations on GC B-cell development and lymphomagenesis. A) The nutrient signaling pathway imposes an 'anabolic capacity' barrier over B-cell activation by T-cell help (and B-cell receptor signaling, not shown), ensuring that growth occurs only if nutrients are sufficient. GC B-cells are clonally selected for the expression of high-affinity antibodies (adequate B-cell competitiveness). **B)** RagC mutations weaken this barrier and cause enhanced B-cell activation, while retaining the ability to suppress activation when nutrient levels are low. These mutations lead to an increased B-cell competitiveness, which translates into enhanced B-cell growth and survival. Upon acquiring additional transforming events, the GC B-cell ultimately develops into a lymphoma. RagC-mutated cells are sensitive to Rapamycin-mediated pharmacological inhibition of mTORC1. Adapted from Fig, 6 of ref. 9.